Journal of Autoimmunity (1993) **6**, 159–167

Effects of Inoculation with Attenuated Autologous T Cells in Patients with Rheumatoid Arthritis

J. M. van Laar, A. M. M. Miltenburg, M. J. A. Verdonk, A. Leow, B. G. Elferink*, M. R. Daha†, I. R. Cohen‡, R. R. P. de Vries* and F. C. Breedveld

*Departments of Rheumatology, *Immunohaematology and Bloodbank, and †Nephrology, University Hospital, Leiden, The Netherlands; ‡Department of Cell Biology, Weizmann Institute of Science, Rehovot, Israel*

(*Received 20 July 1992 and accepted 27 October 1992*)

Injection of attenuated autoimmune T cells, T cell vaccination, has been used successfully in the prevention and treatment of experimental animal autoimmune disease. In order to determine whether such a procedure might be applied in rheumatoid arthritis (RA), a phase I study was conducted in thirteen RA patients with a mean disease duration of 12.8 years. All patients received a subcutaneous injection of attenuated autologous T lymphocytes from a CD4 positive clone ($n=4$) or line ($n=9$) isolated from synovial tissue ($n=3$) or synovial fluid ($n=10$). No toxic side effects were observed. On the average the patients showed a slight decrease in disease activity which was most marked at 8 weeks after the injection. Specific immune reactivity against the injected T cells was not detected, with the possible exception of one patient who was vaccinated with a clone selected *in vitro* with antigen and whose disease had begun one year earlier. In this patient a clear decrease in disease activity occurred, which was associated with a decrease in mitogen-induced proliferation of her peripheral blood mononuclear cells and in titres of serum rheumatoid factors.

The results of this study show that inoculation of RA patients with autologous T cells is technically feasible and non-toxic, and may be associated with clinical and immunological effects. The data suggest that the potential of T cell vaccination should be further explored in diseases with defined antigen reactivity.

Introduction

In animal models T lymphocytes have been used to prevent or treat autoimmune diseases by an intervention called T cell vaccination (TCV). Injection of auto-

Correspondence to: Prof. Dr F. C. Breedveld, University Hospital, Department of Rheumatology, Building 1, C2-Q, P.O. Box 9600, 2300 RC Leiden, The Netherlands.

0896–8411/93/020159+09 $08.00/0

© 1993 Academic Press Limited

immune T cells to syngeneic animals can transfer disease, but if these T cells are activated and rendered avirulent they may prevent subsequent disease induction and ameliorate existing disease. It was demonstrated that autoimmune T cells effective in TCV could be obtained from diseased animals by specific expansion using the autoantigen as an in-vitro stimulus, but also by non-specific expansion using a mitogen [1].

In patients with rheumatoid arthritis (RA) treatments aimed at down regulation of the number or activity of T cells have been relatively successful [2–5]. The goal of this study was to determine the technical feasibility, toxicity and immunomodulatory potential of TCV in RA patients as well as to obtain a preliminary impression of the influence of TCV on disease activity.

Patients and methods

Thirteen patients who fulfilled the established criteria for RA were evaluated. The patients selected included 10 females and 3 males with an average age of 47.9 ± 16.3 years (range 19–70 years) and a mean disease duration of 12.8 ± 12.7 years (range 1–47 years). The patients had active disease and were treated with non-steroidal anti-inflammatory drugs ($n = 12$) and/or disease modifying drugs ($n = 9$). This therapy was left unchanged for 3 months preceding treatment and 6 months thereafter. The patients were followed from 2 weeks prior to 6 months after inoculation. Disease activity was monitored by measuring the Ritchie index [6], the number of swollen joints, the duration of morning stiffness, grip strength, as well as ESR and CRP levels. The clinical and laboratory data of one patient (A1) will be presented separately because of clear changes observed in clinical and immunological parameters. The patient is a woman born in 1955 who developed erosive RA one year prior to treatment. She was treated with salazosulphapyridine 2 gm/day. The protocol was reviewed and approved by the Ethics Committee of the Leiden University Hospital.

Processing of inoculum

Patients were inoculated subcutaneously with in-vitro expanded autologous T cells prepared according to either of the two following protocols (A and B). Group A ($n = 4$) was inoculated with 50×10^6 fixed CD4+ cells of one T cell clone and group B ($n = 9$) with 50×10^6 irradiated cells of one T cell line. The inoculum for group A was prepared from mononuclear cells isolated from synovial fluid (SF-MNC) by Ficoll-Isopaque centrifugation ($n = 3$) or from synovial tissue (ST) as described previously ($n = 1$) [7]. After an initial screening for antigen reactivity by a proliferation assay, a T cell line reactive with the acetone precipitable (AP) fraction of Mycobacterium tuberculosis was established from patient A1 by culturing SF-MNC in 24-well culture plates (Costar 3524, Cambridge, MA, USA) at a concentration 1×10^6 cells/well with 10 μg/ml AP (kindly donated by A. Frenkel, Weizmann Institute of Science, Rehovot, Israel). Following limiting dilution in 96-well plates and cyclic stimulation with AP, irradiated autologous feeder, cells followed by expansion with 50 U/ml rIL-2 (Cetus, Gouda, The Netherlands), rapidly growing AP reactive CD4+ clones were selected for further propagation. Peripheral blood mononuclear cells (PB-MNC) obtained by lymphapheresis served as feeder cells. In patients

A2 and A3, the SF-MNC, and in patient A4, ST-MNC, without defined antigen reactivity were directly cloned by limiting dilution and cyclic stimulation with 10 ng/ml OKT3 monoclonal antibody (mAb) (kindly provided by Cilag, Herenthals, Belgium), 50 U/ml rIL-2 and autologous feeder cells. Rapidly growing CD4+ clones were selected for the preparation of the inoculum.

The inoculum for group B was prepared by expanding the SF-MNC ($n=7$) or ST-MNC ($n=2$) in 24-well plates with rIL-2 to select in-vivo activated cells. After one week the cells were propagated by cyclic stimulation with OKT3 mAb, rIL-2 and autologous feeder cells. Control cell lines were prepared from SF- and PB-MNC by direct stimulation with OKT3 mAb and rIL-2. The control clones were cultured with allogeneic feeder cells.

When 200×10^6 cells were available the cells were activated prior to inoculation, by incubation for 48 hours on OKT3 mAb (0.1 μg/ml) coated plates in the presence of rIL-2. Fifty million cells were either fixed in 0.3% formaldehyde (Baker, Deventer, The Netherlands) (group A) or irradiated with 3,000 rad (group B) and injected subcutaneously in both upper arms.

Immunological monitoring

In-vitro reactivity of PB-MNC against the inoculum and control cells was measured by culturing 1×10^5/well PB-MNC with 1×10^5/well irradiated stimulator cells. Lymphocyte proliferation was assessed by measuring ^{3}H-thymidine (1 μCi of ^{3}H-thymidine) incorporation. Results are tabulated as mean cpm of triplicate cultures. Standard deviations of cpm were less than 15% of the mean. The presence of serum antibodies against the inoculum was assayed by incubation of injected cells with patient serum for 30 minutes on ice. After washing and incubation with FITC-conjugated goat anti-human immunoglobulin, fluorescence was measured by FACS-scan. A standard complement dependent cytotoxicity-assay was also employed. To determine reactivity against the inoculum *in vivo*, patients of group B were challenged intracutaneously with 5×10^6 irradiated cells of the inoculum immediately before and 2 months after treatment. To determine reactivity of PB-MNC against antigens or mitogens after treatment, lymphocyte proliferation was measured as described above after incubation of PB-MNC with 1 μg/ml PHA (Wellcome Foundation, UK), or irradiated allogeneic PB-MNC.

Phenotyping of PB-MNC was performed by flow cytometry using a FACS-scan. Serum rheumatoid factors were determined by ELISA as previously described [8]. Levels of RF are expressed as units compared to a positive standard serum calibrated against the WHO reference serum. Total serum immunoglobulins were determined by Mancini.

Results

Inoculation with attenuated autologous T cells is technically feasible and not toxic

The time needed to produce the inoculum was 3 to 8 months (mean 6.3) for group A and 1 to 6 months (mean 4.3) for group B. The CD4/CD8 ratios of the cell populations prepared to inject group B varied between 0.22 and 40.5. The difference in

culture methods between cells of the inoculum and control lines did not include a consistent change in this ratio (data not shown).

The subcutaneous injection of T cells was well tolerated by all patients. No adverse reactions were documented. One patient developed septic arthritis of a knee prothesis. Leg ulcers were considered to be the *porte d'entrée* of the microorganism.

Inoculation and disease activity

All clinical parameters pointed to a small decrease in disease activity, being most marked at 8 weeks after inoculation. At this time an improvement in the mean values of the Ritchie index (from 12.0 ± 9.5 to 9.5 ± 6.4), number of swollen joints (from 8.5 ± 4.8 to 6.8 ± 4.8), grip strength (26.8 ± 19.2 kPa to 29.0 ± 20.8 kPa) and duration of morning stiffness (from 51.2 ± 65.9 min to 33.8 ± 44.1 min) was observed. The mean ESR (from 48.9 ± 36.6 mm/hr to 45.5 ± 30.8 mm/hr) and mean CRP (from 39.9 ± 31.7 mg/l to 26.8 ± 20.6 mg/l) also decreased in this time period. All parameters except the Ritchie index had returned to pretreatment levels after 6 months. Repeated measures-analysis of variance showed that only the decrease in Ritchie index was statistically significant (time effect $P = 0.01$). The changes in the clinical parameters between the patients of groups A and B did not differ significantly. The changes in clinical parameters observed in the complete patient group and those of patient A1 are depicted in Figure 1.

Responses against the injected cells

Responses of PB-MNC were determined against activated and resting cells of both the inoculum and control cells. Activated and resting cells were either fixed or irradiated before being used as stimulator cells. Significant pretreatment responses (3,000–8,000 counts/min) against activated irradiated cells of the inoculum, but also of control T cells, were measured in all patients tested (three of group A and six of group B). No or low responses ($< 5{,}000$ counts/min) were detected against resting or fixed T cells. Except for patient A1 no clear change in response was observed after treatment. In patient A1 enhancement of the response to the injected clone was observed on day 2; at day 5 this response had subsided to pretreatment levels. This enhancement was not observed for a control clone not reactive with AP (Fig. 2).

Intradermal challenge with cells of the inoculum was performed in the patients of group B immediately before and 2 months after treatment. Most reactions were maximal after 24 h and did not reach the minimal induration size of 5 mm generally accepted for a positive delayed type hypersensitivity skin reaction. There was no evidence for increased skin test reactivity after inoculation. Antibodies against T cells of the inoculum in serum samples obtained at different times after inoculation were not detected by FACS analysis or by cytotoxicity.

Immunosuppressive effects

No consistent changes in cellular immune reactivity were observed after treatment, but in patient A1 a sharp decrease in PHA reactivity of PB-MNC occurred after

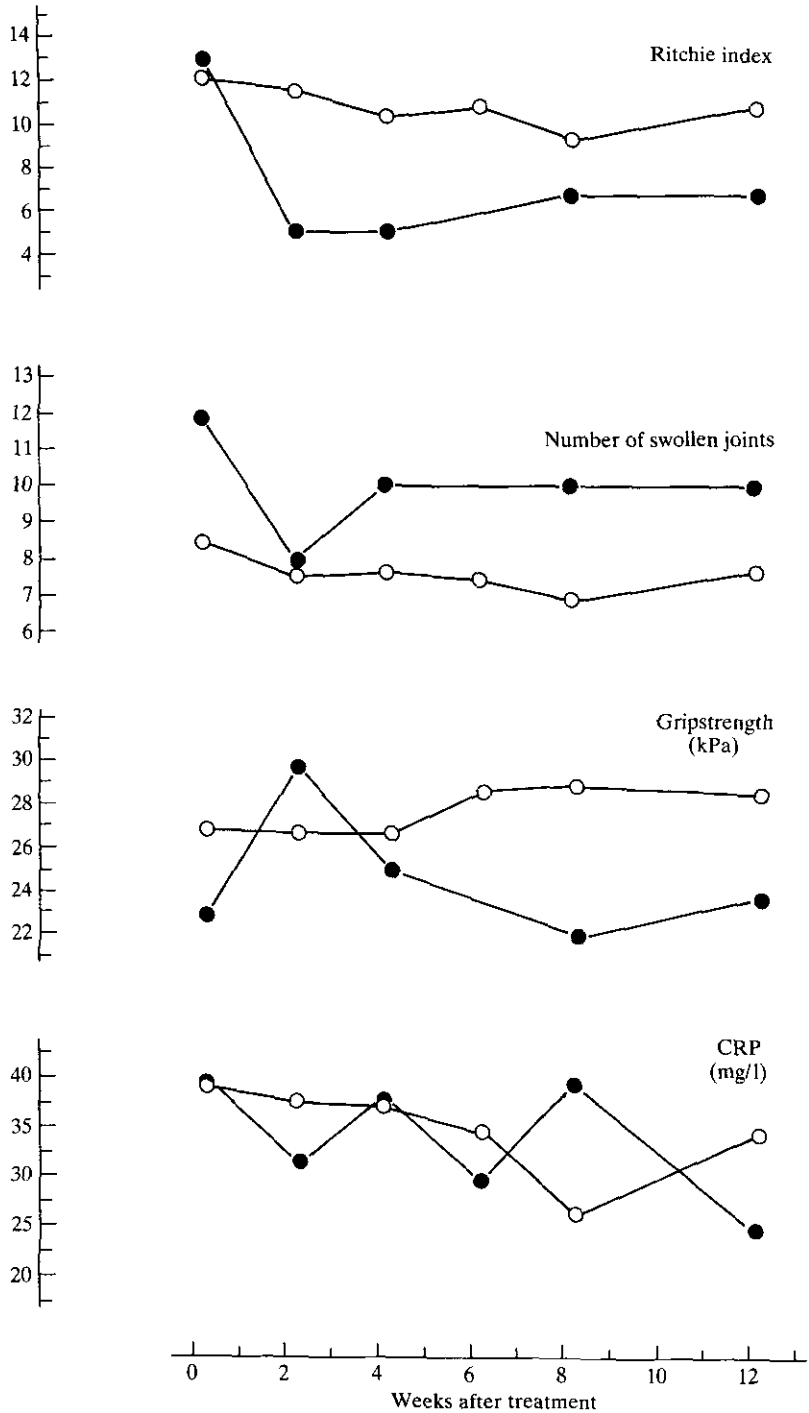

Figure 1. Effects of inoculation with attenuated, autologous T cells on clinical parameters of disease activity observed in 13 patients with rheumatoid arthritis (—○—) and in patient A1 (—●—) after inoculation with attenuated autologous T cells. Standard deviations of all parameters ranged between 55–130% of the mean values (not depicted).

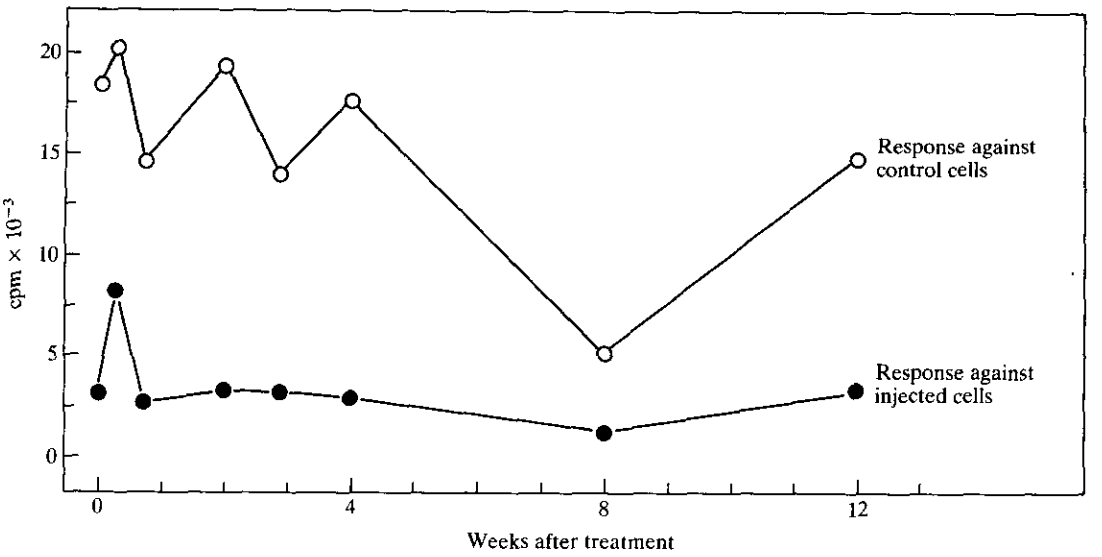

Figure 2. In-vitro proliferative responses of PB-MNC from a patient with rheumatoid arthritis (patient A1) against the injected cells and control cells after treatment with attenuated, autologous T cells. Activated and irradiated stimulator cells were used.

inoculation, whereas the proliferation on stimulation with alloantigens was not influenced (Fig. 3A).

Serum titres of IgM- and IgG-RF also decreased significantly after inoculation of patient A1. This decrease did not reflect a decrease in serum immunoglobulin levels (Fig. 3B). A similar specific decrease in serum RF levels was observed in 2 of the other 6 patients with increased levels of RF isotypes.

To investigate whether the inoculation induced shifts in T cell subsets, phenotypes of PB-MNC were followed longitudinally at 8 time points after treatment. No consistent change in the percentage of CD4, CD8 or memory (CD45RO) vs naive (CD45RA) T cells occurred.

Discussion

In this study the applicability and efficacy of inoculating RA patients with attenuated autologous T cells were explored. This procedure, designated T cell vaccination, has been successfully applied in the treatment of experimentally induced arthritis and other animal models of autoimmune diseases [9]. A phase I study in multiple sclerosis patients had shown this to be a non-toxic procedure [10]; therefore we considered that a study in RA patients was warranted. A similar study in RA patients was recently performed in Mainz [11]. Although the pathogenic T cells have not yet been identified in RA, it was reasoned that these cells would be present in the joint and could therefore be used as potential vaccines for the treatment of disease.

The results show that such a procedure is technically feasible. It was possible to produce an inoculum for all patients invited to participate in the study although the time required was on average 5 months. Both cell yield and rate of in-vitro growth influenced the time needed to produce the inoculum. Hyporesponsiveness of synovial T cells to IL-2 and the use of autologous feeder cells and serum to expand

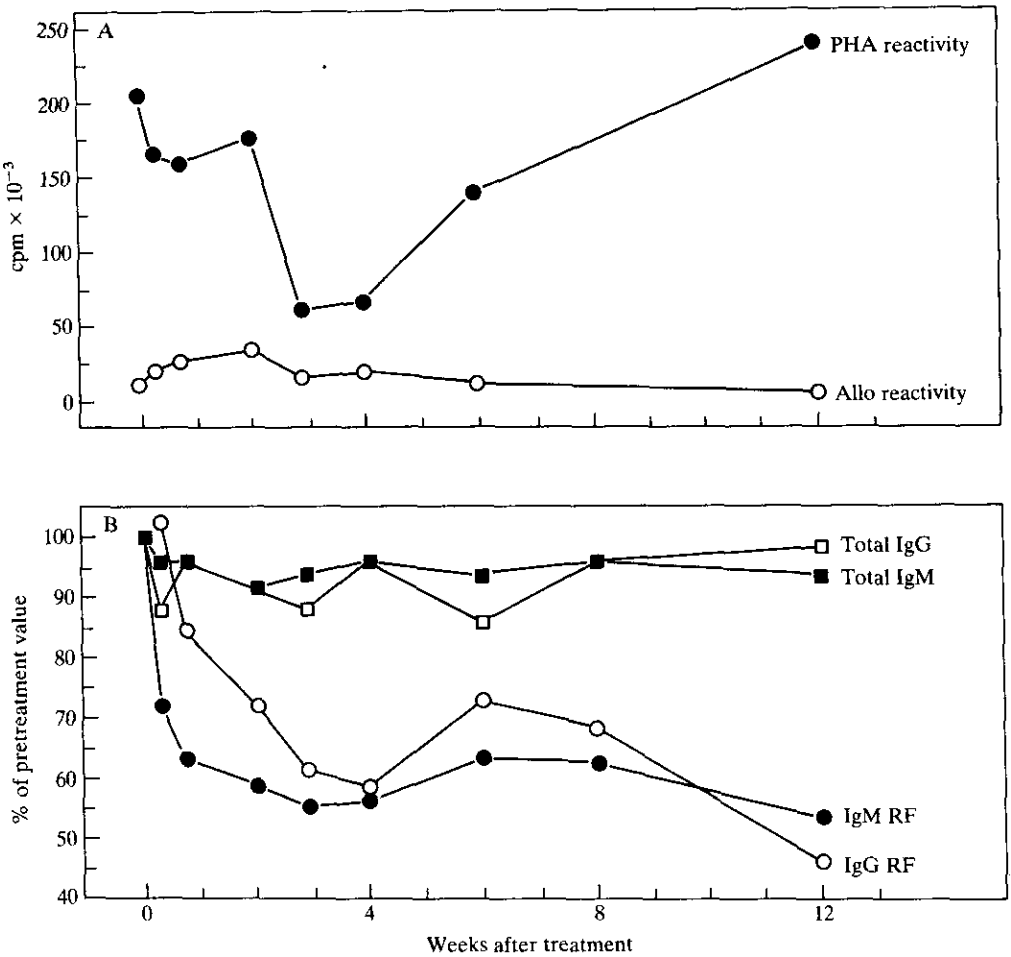

Figure 3. (A) PHA and alloreactivity of PB-MNC from a patient with rheumatoid arthritis (patient A1) and (B) serum titres of IgG- and IgM-RF as well as total serum IgG and IgM in a patient with rheumatoid arthritis (patient A1) after treatment with attenuated, autologous T cells.

the cells used for inoculation may have contributed to the slow growth *in vitro* [12, 13].

A central question in this study was whether the inoculation could induce an immune response that down-modulates the activity of T cells. A humoral anti-T cell response could not be demonstrated. In-vitro proliferative responses of PB-MNC against the activated cells of the inoculum were detected in all patients. However, except for the short lasting increased response of patient A1, there were no indications that such responses were influenced by the inoculation. Nor did the similar results of skin tests for anti-T cell reactivity before and after inoculation suggest the induction of an immune response. The responses found may be attributed to accessory molecule and cytokine mediated interactions of activated T cells and monocytes acting in concert to induce proliferation or influx of resting T cells [14]. Nevertheless, the data on serum RF levels and mitogen reactivity of PB-MNC from patient A1 suggested that the treatment in this patient had immunomodulatory potential.

Experiments in animal models have shown that the proper selection of T cells is essential for effective vaccination. Although the immunization protocol used in RA patients was extrapolated from animal studies, possibly relevant differences remained. Most studies in animals used T cells isolated from lymph nodes of recently immunized animals. In addition these T cells were usually activated by a disease-relevant antigen in the presence of syngeneic antigen presenting cells. Furthermore, the optimal procedures to immunize animals differed between the studies. The number of cells injected, route of administration and fixation or irradiation may all be critical for effective TCV [15].

The RA patients were injected with T cell clones which would allow optimal monitoring of specific T–T cell interactions or with lines from in-vivo activated T cells which would be more likely to contain disease-relevant cells. Neither technique was associated with an immune response against the injected cells, whereas the immunosuppressive effects were heterogeneous and unrelated to clinical effects. The small decrease in clinical and laboratory parameters of disease activity in the whole patient group could well be a placebo effect. Patient A1 was exceptional because of a possible boost of anti-T cell responses after inoculation and because of the immunosuppressive effects which were associated with a clear decrease in disease activity. Furthermore, this patient had a relatively short disease duration and was injected with an antigen reactive clone. If the clinical and immunological changes observed in this patient were indeed induced by the inoculation of T cells, then this observation points to a potential therapeutic effect in an early phase of RA when autoreactive T cells are supposed to play an important role.

This study raises important questions both with respect to TCV in animals and to further research in humans. While some of the issues discussed above can be investigated in animals, further studies in man with a focus on the immunological effectiveness of TCV are also required. The data suggest that the potential of TCV to induce immunosuppression should be explored in diseases with defined antigen reactivity of disease inducing T cells. Such an approach would also allow the monitoring of T cell autoreactivity and the immunomodulatory potential of TCV. Once this has been established, TCV as a potential treatment of human autoimmune disease can be further investigated. The experience obtained in this phase I study may thus serve as a lead for future research in this intriguing field.

Acknowledgements

The detailed clinical and immunological data from this study will, upon request, be kindly provided by the authors. The authors would like to acknowledge the assistance of the European Economic Community Concerted Action on Immunopathogenesis and Immunotherapy of Chronic Arthritis and the European Medical Research Council on Clinical Collaboration on T Cell Vaccination.

References

1. Mor, F., A. W. Lohse, N. Karin, and I. R. Cohen. 1990. Clinical modeling of T cell vaccination against autoimmune disease in rats: selection of antigen-specific T cells using a mitogen. *J. Clin. Invest.* **85:** 1594–1598

2. Paulus, H. E., H. I. Machleder, S. Levine, D. T. Y. Yu, and N. S. Macdonald. 1977. Lymphocyte involvement in rheumatoid arthritis: studies during thoracic duct drainage. *Arthritis Rheum.* **20:** 1249–1262
3. Kotzin, B. L., S. Strober, E. G. Engleman, A. Callin, R. T. Hoppe, G. S. Kansas, C. P. Terrell, and H. S. Kaplan. 1981. Treatment of intractable rheumatolid arthritis with total lymphoid irradiation. *N. Engl. J. Med.* **305:** 969–975
4. Trentham, D. E., J. A. Belli, R. J. Anderson, J. A. Buckley, E. J. Goetzl, J. R. David, and F. K. Austen. 1981. Clinical and immunologic effects of fractionated total lymphoid irradiation in refractory rheumatoid arthritis. *N. Engl. J. Med.* **305:** 976–982
5. Karsh, J., S. H. Klippel, P. H. Plotz, J. L. Decker, D. R. Wright, and M. W. Flye. 1981. Lymphapheresis in rheumatoid arthritis: a randomized trial. *Arthritis Rheum.* **24:** 867–873
6. Ritchie, D. M., J. A. Boyle, J. M. McInnes, M. K. Jasani, T. G. Dalakos, P. Grieveson, and W. W. Buchanan. 1968. Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. *Q. J. Med.* **37:** 393–406
7. van Laar, J. M., A. M. M. Miltenburg, M. J. A. Verdonk, M. R. Daha, R. R. P. de Vries, P. J. van den Elsen, and F. C. Breedveld. 1991. Lack of T cell oligoclonality in enzyme-digested synovial tissue and in synovial fluid in most patients with rheumatoid arthritis. *Clin. Exp. Immunol.* **83:** 352–358
8. Otten, H. G., M. R. Daha, H. H. de Rooy, and F. C. Breedveld. 1989. Quantitative detection of class-specific rheumatoid factors using mouse monoclonal antibodies and the biotin/streptavidin enhancement system. *Br. J. Rheumatol.* **28:** 310–316
9. Cohen, I. R. 1986. Regulation of autoimmune disease, physiological and therapeutic. *Immunol. Rev.* **94:** 5–21
10. Hafler, D. A., I. R. Cohen, D. S. Benjamin, and H. L. Weiner. 1992. T cell vaccination in multiple sclerosis; a preliminary report. *Clin. Immunol. Immunopathol.* **62:** 307–313
11. Lohse, A. W., N. Bakker, E. Hermann, T. Poralla, M. Jonker, and K. H. Meyer zum Büschenfelde. 1993. Induction of an anti-vaccine response by T cell vaccination in primates and humans. *J. Autoimmunity* **6:** 121–130
12. Lotz, M., C. D. Tsouvas, C. A. Robinson, C. A. Dinarello, D. A. Carson, and J. H. Vaughan. 1986. Basis for defective responses of rheumatoid synovial fluid lymphocytes to anti-CD3 (T3) antibodies. *J. Clin. Invest.* **78:** 713–721
13. Rappaport, R. S. and G. P. Dodge. 1982. Prostaglandin E inhibits the production of human interleukin-2. *J. Exp. Med.* **155:** 943–948
14. Kawakami, K., Y. Yamamoto, K. Kakimoto, and K. Onoue. 1989. Requirement for delivery of signals by physical interaction and soluble factors from accessory cells in the induction of receptor-mediated T cell proliferation. *J. Immunol.* **142:** 1818–1825
15. Beraud, E., O. Lider, E. Baharav, T. Reshef, and I. R. Cohen. 1989. Vaccination against experimental autoimmune encephalomyelitis using a subencephalitogenic dose of autoimmune effector cells. (1) Characteristics of vaccination. *J. Autoimmunity* **2:** 75–86